EXPERIMENTS ON THE DETERMINATION AND DIFFERENTIATION OF SEX IN THE BOPYRID STEGOPHRYXUS HYPTIUS THOMPSON[1]

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One of the great controversies related to the Bopyridae (a family of isopod crustaceans, suborder Epicaridea), and one that has broader biological implications as well, is the question of sex-determination. Is sex already determined in the larval stage, or does the fate of the larva (i.e. whether it turns into a female or a male) depend on environmental influences? Can such external factors as the position the larva occupies or the nourishment it receives bring about sex reversal in an already sex-determined larva?

The chief views expressed in the past regarding this question are as follows:

1. Giard and Bonnier (1887) maintained that in the Bopyridae all free-swimming larvae are males. The first larva, however, that invades a particular host undergoes sex reversal and transforms into a large female without ever having functioned as a male. The next to come settles on this female and metamorphoses into an adult dwarf male which fertilizes the former.

2. Smith (1909) and Goldschmidt (1920) stated that all the species of Epicaridea, Bopyrina as well as Cryptoniscina, are protandric hermaphrodites, each individual being male while in a larval state, and then losing its male organization and becoming female as the parasitic habit is assumed. The females, therefore, result from males that have already functioned as males.

3. Hiraiwa (1936) believed that the free-swimming larvae are not males but are sexually undifferentiated, although the sex is already predetermined. Differentiation follows fixation, but is probably not due to environmental factors.

4. Recently, Caullery (1941), impressed by the influence of association on sexuality as exhibited in such animals as Bonellia, Crepidula, and Ophyotrocha, made the suggestion that the sexes may not be fixed from the start, but that direct parasitism of a larva on a host entails differentiation into a female, and indirect parasitism, through the intermediary of a female on which it is stationed, entails differentiation into a male. Lacking direct evidence, however, he suggested an experimental approach to test the validity of this theory. He advised collecting the newly-arrived cryptoniscid larvae that can frequently be found in the brood pouch of a female bopyrid—larvae which according to this view would evolve into typical males under the influence of the environment—and placing them in contact with young crabs not yet parasitized. Caullery thought it probable that these larvae would fix to the crab and become females.

These suggestions of Caullery moved the writer to undertake a series of experiments with the larvae of *Stegophryxus hyptius* Thompson, an ectoparasite of the

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hermit crab *Pagurus longicarpus* Say. This work was carried on in the summer of 1946 at the Marine Biological Laboratory, Woods Hole, Mass. In addition to the experiment suggested by Caullery, reciprocal experiments were undertaken in which presumptive female larvae were taken from the host crab and transferred to the brood pouch of a female bopyrid to test the suspected masculinizing influence of the female on cryptonisci that attach to her body.

The present paper reporting on this work was ready for publication when the writer's attention was drawn to an article in Italian by Reverberi and Pitotti, which, although it appeared in 1942, had not been mentioned in the abstracting journals until 1947. This paper provides the first experimental verification of any of the proposed sex-determination theories with reference to the Bopyridae. The authors, working with *Ione thoracica* Montagu, showed that the control of sex-determination is environmental rather than genetic. However, since there are several points of difference between the biological cycles and sex phenomena of Ione and Stegophryxus, it was decided not to alter the present paper as originally written, but in the discussion and footnotes to draw a comparison between the results reported by Reverberi and Pitotti and our own.

Life Cycle of Stegophryxus

Only about 1.5 per cent of *Pagurus longicarpus* at Woods Hole are parasitized by *Stegophryxus hyptius*. Thompson (1901), in his original description of the species, gave an account of the morphology of the adult female, adult male and some of the immature forms, but the life cycle has heretofore not been discussed.

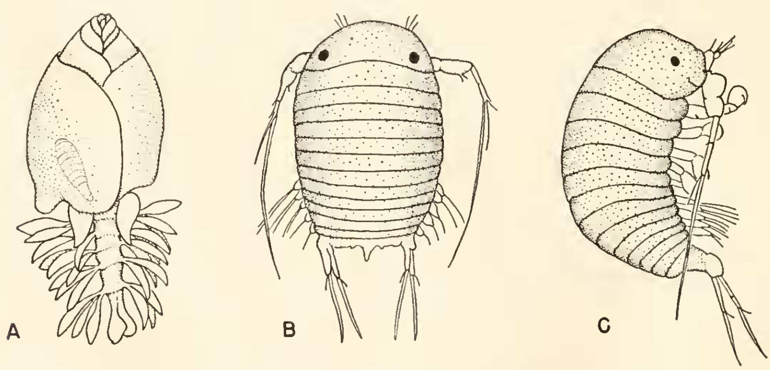

FIGURE 1. Adult female and epicaridium larva of *Stegophryxus hyptius*.
A. Ventral view of adult female. The dwarf male, although not visible externally, is shown within the brood pouch by a dotted outline to indicate its position and relative size. × 5. B. The epicaridium or first larval stage shown in dorsal view. × 120. C. Lateral view of epicaridium larva. × 120.

The female Stegophryxus, as is the case in all bopyrids, is much larger than the male (Fig. 1A). It occurs on the abdomen of the hermit crab, to which it is attached, back downward, by its mandibles and legs. Its thorax is concealed ventrally by an enormous brood pouch, made up of five pairs of thin brood plates.

Within this brood pouch lies the slender dwarf male, whose function is not that of inseminating the female and then quitting her, but of remaining in readiness to fertilize the successive batches of eggs that are released into the brood pouch during the female's productive life. These eggs, within two weeks after fertilization, develop into first stage larvae that leave the mother and swim off. After an interval of about five days, the marsupium is again filled with eggs and another brood begins embryonic development.

The first larval stage is known as the *epicaridium*. It is a short, broad, semi-barrel-shaped larva (Fig. 1B) with sub-chelate pereiopods for clinging and with pleopods in the form of swimming organs. The epicaridium of Stegophryxus measures about 270 μ in length, 150 μ in breadth, and 120 μ in depth (not including the appendages). In this stage the young of Stegophryxus escape from the brood pouch and swim off as plankton organisms. In the laboratory, they quickly rise to the surface of the water and remain there floating or swimming about for days.

The subsequent history of the epicarid larva has not been investigated in Stegophryxus. It may attach to a pelagic copepod, undergo a molt, and become a *microniscus* larva, which, after feeding on the copepod, will eventually transform into a new larval stage known as the *cryptoniscus* that swims off to seek the definitive host. This type of development is known to occur in some of the Epicaridea (Sars, 1899; Caullery, 1907; Caroli, 1928; Reverberi and Pitotti, 1942). Or the epicarid larva may develop directly into a cryptoniscus larva, an abbreviated type of development which Hiraiwa (1936) believes is the case in most Bopyridae. We postulate the first alternative in the case of Stegophryxus because of the great difference in size between its epicaridium and cryptoniscus stages, a difference which can only be accounted for by assuming the existence of an intervening stage.

At any rate, however arrived at, the earliest larval stage of Stegophryxus that we find on the crab is the cryptoniscus. In this stage (Fig. 2) the parasite is typically isopod in its characteristics. It has an elongated body, dorsoventrally compressed, segmented and well chitinized, There are seven pairs of thoracic appendages (as compared with six pairs in the epicaridium) all similar in form, six pairs of uniramous natatory pleopods, and one pair of biramous uropods. The cryptoniscus measures about 680 μ in length, being therefore about two and one-half times longer than the epicaridium.

We have been able to distinguish three phases in the life of the cryptoniscus larva on the basis of color pattern which we shall designate as (1) the brown chromatophore phase, (2) the black chromatophore phase, and (3) the striped contracted phase.

The youngest cryptonisci, those that have recently settled on a crab, have a profusion of dark brown expanded chromatophores that cover the dorsal surface of the body in such a way as to leave an uncolored portion that resembles a cross (Fig. 2A). These chromatophores are present laterally on the head and segments 1, 4, 5, 6, 7, and 8; are present centrally as well as laterally on segments 12 and 13 and on the uropods; and are entirely absent from segments 2, 3, 9, 10, and 11. The general body color is pale yellowish and is due to another system of chromatophores, which are scattered over the integument without definite plan. The eyes are reddish brown.

In phase 2, the light colored cross-shaped pattern remains much as before, but most of the areas formerly occupied by brown chromatophores are now occupied

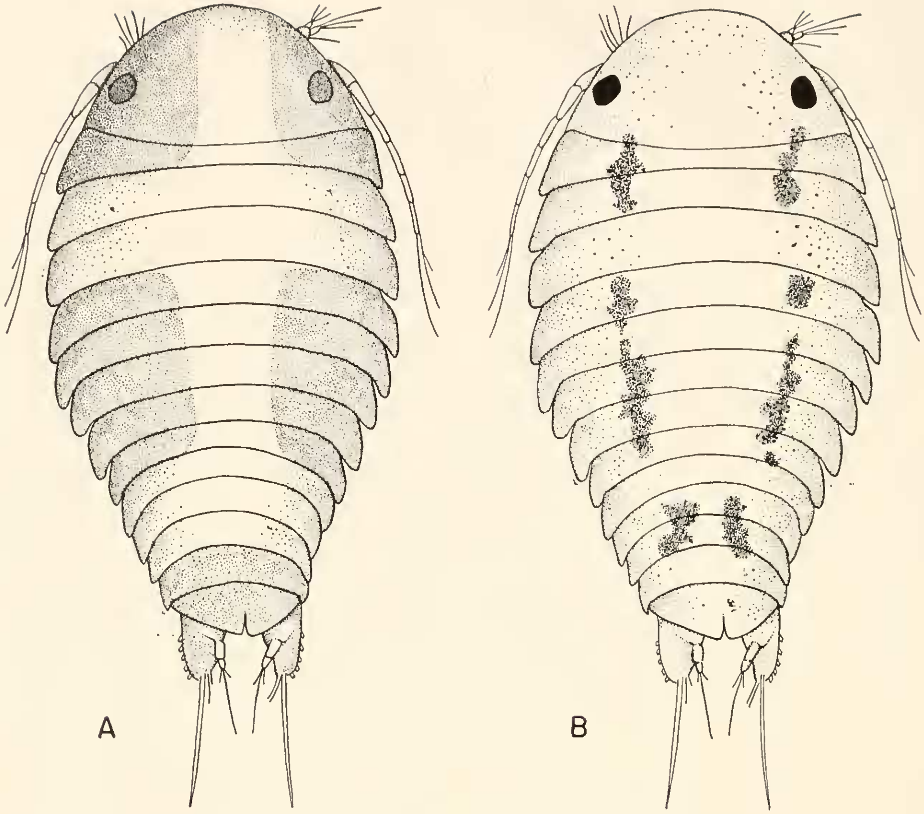

FIGURE 2. The cryptoniscus larva of *Stegophryxus hyptius.*

A. Young larva showing color pattern when in phase 1. The light cross-shaped area is devoid of chromatophores. B. Older cryptoniscus in phase 3 with contracted melanophores forming an interrupted stripe on each side.

by expanded black chromatophores. Their distribution is as follows: present laterally on the head and segments 1, 4, 5, 6, 7, and 8; present centrally on segments 11 and 12; absent from segments 2, 3, 9, 10, 13 and the uropods. The eyes have also become black. The yellow chromatophores are now more noticeable and have become restricted to segments 1 to 11 inclusive where they are present laterally.

In phase 3 (Fig. 2B) the black chromatophores are much fewer in number and are all in the contracted state. They form a broken chain on each side of the body about midway between the center and margin of the dorsal surface, reaching from segment 1 to segment 8 inclusive, but absent on segment 3. On segments 10 and 11 there are a few black chromatophores centrally located. Yellow chromatophores are intermingled with the black in the same chain but extend from segment 1 to segment 11. The eyes are black. In this stage the cryptoniscus is ready for the molt which will transform it into a juvenile female of the first postlarval stage.

No structural differences have been detected in these three cryptoniscid stages. Since neither the brown nor the black chromatophores lose their color in alcohol, they are no doubt melanophores which presumably differ only in the amount of melanin present.

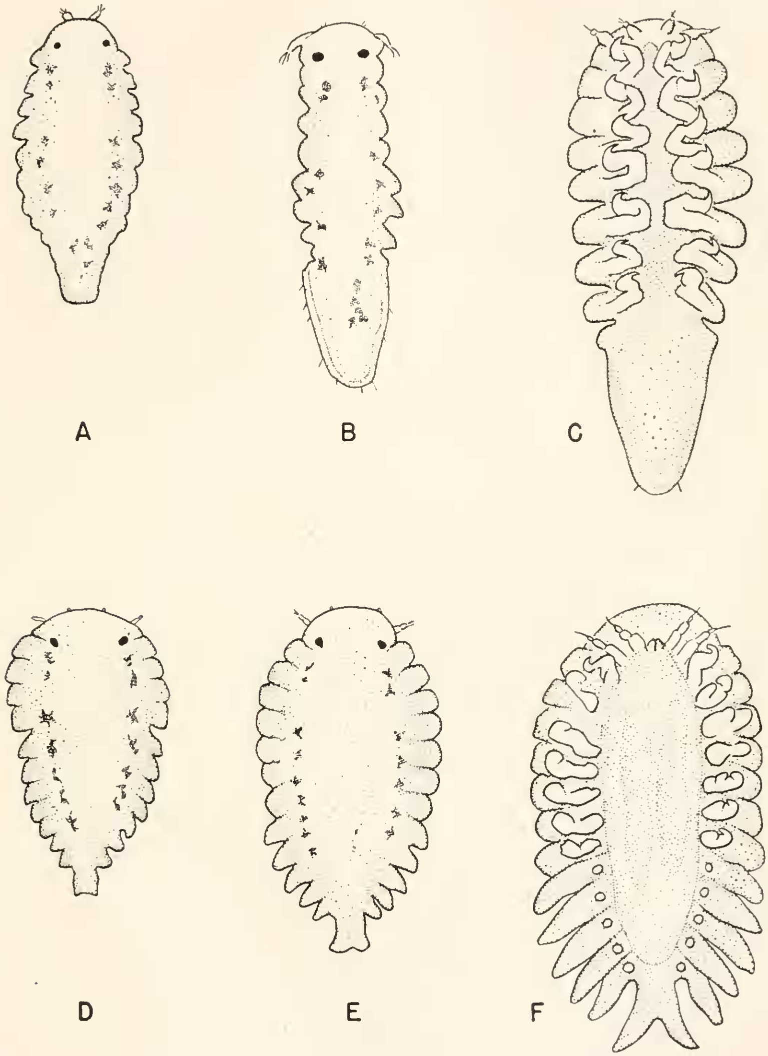

FIGURE 3. Juvenile males of *Stegophryxus hyptius* compared with juvenile females of the same species and same degree of development.

A. Juvenile male in first post-cryptoniscid instar. Specimen drawn measured 0.85 mm. Dorsal view. B. Older juvenile male measuring 1.07 mm. in length. Dorsal view. C. More advanced juvenile male measuring 1.4 mm. in length. Ventral view. D. Juvenile female in first post-cryptoniscid instar. Specimen drawn measured 0.85 mm. Dorsal view. E. Older juvenile female, 1.01 mm. in length. Dorsal view. F. More advanced juvenile female measuring 1.3 mm. in length. Ventral view.

The juvenile female into which the cryptoniscus transforms is shown in Figure 3D. It is broader than the cryptoniscus and the pleon lacks uropods and possesses only stump-like rudiments of pleopods. The terminal segment is extended into a tail-like outgrowth ending in a shallow notch. The animal is whitish with color pattern differing little from that of the last stage cryptoniscus. More advanced juvenile females are shown in Figures 3E and 3F.

About ten per cent of the crabs examined in the summer of 1946 were infested with one or more cryptonisci. This was fortunate, insofar as experimental possibilities were concerned, since infective cryptonisci have rarely been reported. Bonnier, for example, having studied about eighty species, came across cryptoniscus larvae that had recently attached to the host only twice. Hiraiwa never found them during five years' study of *Epipenaeon japonica*. The cryptonisci of Stegophryxus may be found on almost any part of the crab, but only those attached to the pleopods have actually settled down. The others are transients or new arrivals that wander off at the slightest disturbance. Even those on the pleopods, while more permanent than the others, are apt to leave when disturbed. The older the cryptoniscus, the more fixed in position it is, and if found attached to the last pleopod (third abdominal appendage of males, fourth of females), the favorite resting site, one can presume that it will remain there, barring accidents, until it eventually transforms into a female. Only after the juvenile female stage is assumed does the parasite leave the pleopod to fix itself permanently on the abdomen proper of the host.

Cryptonisci destined to become functional males are those found attached to a young or mature female. They are identical in form, size, and color pattern with those found on the crab. They too pass through the same three phases, but metamorphose into juvenile males. The first male instar is shown in Figure 3A. It is narrower in form than the corresponding female instar and has a strikingly different type of pleon which is tongue-shaped and rounded at the tip. More advanced juvenile males are shown in Figures 3B and 3C.

Transformation of Presumptive Females Into Males

The first question to be answered experimentally was whether or not the cryptonisci found on normal crabs, that is on crabs not infested with a female Stegophryxus, could be transformed into males. Such larvae in all likelihood would be presumptive females. If removed from the crab and transferred to the brood pouch of a female bopyrid, would these cryptonisci metamorphose into males?

I. In the first series of experiments, cryptonisci taken at random from normal crabs were placed in a dish with a crab parasitized by a mature Stegophryxus. The male was first removed from the brood pouch of the female to prevent interference. This was necessary because if the adult male is allowed to remain, although cryptonisci will attach as freely as when no male is present, they will enjoy only a relatively brief period of attachment before they are driven off.

Four experiments of this type were conducted. In all cases the greater proportion of the cryptonisci attached to the female bopyrid and the greater proportion likewise entered the juvenile male phase. But, after varying lengths of residence in the brood pouch and correspondingly varied degrees of attainment of the male phase, all but one out of each lot eventually deserted the female. The one that remained in unchallenged possession eventually became a mature male, and in cases where the experiment was continued long enough this male functioned as such and successfully fertilized the eggs of its consort which then developed normally into epicaridium larvae.

It will be sufficient to cite one experiment of this series in detail. This experiment was begun July 13, 1946 with five cryptonisci taken at random from unparasitized crabs and placed in a dish with a crab having a mature Stegophryxus (male removed) whose brood pouch contained late embryos.

July 14. Two cryptonisci have attached to the female Stegophryxus.

July 15. Four cryptonisci now present on the bopyrid. Epicarids are hatching.

July 16. Three cryptonisci remain within the now empty brood pouch. They have developed to the black pigment stage.

July 17. Metamorphosis of cryptonisci continuing; one, at least, has molted.

July 19. The three cryptonisci have entered the juvenile male phase and one is slightly more advanced than the others.

July 24. The three juvenile males are still present and continuing their development.

July 26. One of the juvenile males has disappeared. One of the two remaining ones is permanently removed for examination and drawings are made of it.

August 9. The brood pouch of the female bopyrid is now filled with eggs. (This means that the male has reached maturity.)

August 11. The male was removed for measuring and returned to the brood pouch. Its length is 2.28 mm.

August 24. Development of the eggs has continued normally and today the epicarid larvae are released.

August 26. The male now measures 2.37 mm. Experiment discontinued.

Similar results were obtained when female bopyrids, found in nature with a retinue of cryptonisci present in the brood pouch, were kept under observation. In one case a female Stegophryxus, non-ovigerous and lacking a male, had 18 cryptonisci attached to it. The daily count showed a reduction as follows: 18, 16, 14, 11, 9, 6, 4, 2, 2, 2, 2, 2, 2, 1. The remaining one reached maturity two weeks later and fertilized the eggs of the female which were not released until that time. Another reduction from an initial natural retinue of eight cryptonisci occurred as follows: from 8 to 2 in four days, but these two persisted for 11 more days to become juvenile males, then one disappeared. The survivor became a mature male.

These experiments and observations, while they shed some light on the problem at hand, are inconclusive evidence for or against any theory of the sexual nature of the cryptonisci. They show that cryptonisci that enter the brood pouch of a female metamorphose in the male direction, but what of those that leave early or fail to enter? Could not they be predetermined females unresponsive to masculinizing influences?

II. To settle this point, it was decided to experiment with single cryptonisci. Moreover, only cryptonisci found clinging to the posterior pleopod of a normal crab were used. Nine experiments were undertaken. In five of these the cryptoniscus selected for insertion in the brood pouch of a female was in the brown chromatophore stage; the four other cryptonisci were in the more advanced black stage.

Each of the five Stage 1 cryptonisci remained in the brood pouch and made no efforts to crawl out. One was removed after six days, one after eight, one after eleven, and two after twelve days. Each one had metamorphosed into a male, whose size and extent of development was proportional to the length of time spent in the brood pouch. Those that had been on the female for eleven or twelve days had reached a size of from 1.5 mm. to 1.8 mm.

The experiments with Stage 2 cryptonisci gave different results. In three cases the cryptoniscus crawled out of the brood pouch within a day or two and was either lost or found clinging to the crab instead. One experiment yielded positive results. This cryptoniscus refused repeatedly to attach to the female, but after each escape it was returned to the brood pouch. Finally it remained there, and, eventually, 28 days later, had become a 2 mm. male.

The positive results obtained with the five Stage 1 cryptonisci strongly indicate that cryptonisci that would ordinarily become females can readily be transformed into males through attachment to the body of the female bopyrid, provided the transfer is effected at an early age. Even the one success with a Stage 2 cryptoniscus confirms this. It must be concluded that after a certain period of parasitism on the crab the cryptoniscus becomes female-determined and the direction of its sex development can no longer be changed under ordinary conditions. Subsequent experiments, using juvenile females for transfer, instead of cryptonisci, support this view and will now be briefly recounted.

III. Four attempts were made to transform juvenile females into males. All were completely unsuccessful. Juvenile females in the early post-cryptoniscid phase were used, before they had developed far enough to leave the pleopod of the host for permanent attachment on the abdomen.

One female remained inside the brood pouch for one day, crawled to the exterior and remained there one day, then disappeared.

One left the brood pouch the day after transfer and attached to the abdomen of the crab where it remained for eight days, when the crab died.

One left the brood pouch on the second day and attached to the underside of the telson of the crab. It remained there until the experiment was discontinued 12 days later, and grew from an initial size of .85 mm. to 1.4 mm.

The fourth one was transferred to the brood pouch together with the pleopod to which it was attached. This female remained for five days, then disappeared and could not be recovered for examination.

It would seem that juvenile females are averse to becoming ectoparasites of other more mature females. They leave such an unnatural situation to return to direct parasitism on the crab.[2] There is no evidence that any of the four were modified by their brief sojourn in the brood pouch of another female.

[2] Reverberi (1947) came to the same conclusion with regard to Ione. However, he then placed two females together *in vitro* apart from the host, one being a juvenile female and the other an adult from which the juvenile would have to derive its nourishment. As often as the adult died, another of the same age would be substituted. By this ingenious method he was able to maintain a direct association between a juvenile female and an adult Ione for several months. One case of definite sexual inversion resulted from many trials of this sort. This particular juvenile female underwent external changes and gradually took on the appearance of a male. When killed and sectioned after nearly four months under these experimental conditions, the individual was found to have normal testes partially filled with sperm.

Attempts to Transform Presumptive Males into Females

If presumptive female cryptonisci can be turned into males by altering the environment, the question naturally arises regarding the possibility of producing females from presumptive male cryptonisci. The method of experimentation would be to remove cryptonisci from the brood pouch of a female bopyrid and transfer them to a crab instead. This is the type of experiment on which Caullery pinned his hopes of verifying the theory of sex determination in Epicaridea based upon the type of association with the host.

When this was tried it invariably led to failure because the transferred cryptonisci did not remain attached to the crab long enough to show either positive or negative results. This failure to remain attached need not necessarily be attributed to aversion on the part of the cryptonisci for a strange environment. Indeed, the hazards in the case of direct parasitism on the crab are great. When it is recalled that approximately 10 per cent of the normal crabs have cryptonisci on their surface and only 1.5 per cent of all crabs are infested with female Stegophryxi, it becomes clear that many potential parasites are eliminated through environmental difficulties. Moreover, no success was achieved in numerous attempts to rear to the juvenile female stage cryptonisci found naturally attached to crabs. When crabs bearing cryptonisci are isolated in a dish and examined after a day or two, one finds that the cryptonisci have disappeared. Apparently they are eaten by the crab, since cryptonisci kept in dishes without crabs will remain alive for as long as two weeks. It may be remarked in passing that although isolated cryptonisci survive, they do not develop, nor pass from the brown to the black phase. It is significant that the juvenile female Stegophryxi naturally occurring on crabs can be reared without difficulty in the laboratory. They are actually fixed to the crab and have lost their ability to swim, whereas the cryptonisci, as explained before, are still active and only perch on the crab without fastening themselves to it. Should they disengage themselves even momentarily, they are in danger of being caught up by the currents passing through the gill chambers of the crab and swept in the direction of the crab's mouth. The mouth parts of the crab are in constant motion and any particle that comes in contact with them is trapped and masticated.

The failure of these experiments with cryptonisci removed from the brood pouch of a female and transfered to a crab may, therefore, with considerable assurance, be laid to experimental difficulties.[3] When the proper technique is worked out for Stegophryxus, which will eliminate the hazards facing cryptonisci that attach to crabs, we feel confident that presumptive male cryptonisci can be transformed into females.

[3] This is especially reasonable in view of the fact that Reverberi and Pitotti (1942) experienced a similar lack of success when they tried to implant the cryptoniscus larvae of Ione on the gills of Callianassa. The cryptonisci invariably failed to remain on the host (pp. 148–149). But when they used post-cryptoniscid stages they were successful in bringing about the transformation of juvenile males into females. Ione, unlike Stegophryxus, is a branchial parasite. When juvenile males of Ione, removed from adult females, were placed in the branchial cavity of the host, they soon attached to the branchiae, began to feed, and in the majority of cases remained there more or less permanently. Such males gradually became females.

The Fate of Supernumerary Males and Females

The first series of experiments reported above furnish evidence that although any number of cryptonisci may attach to the same female and develop into juvenile males, only one male is allowed to reach maturity. This point has not been realized by most previous investigators except Caullery (1941) and Reverberi and Pitotti (1942), and hence, in the older literature, several cases of supernumerary males associated with one female bopyrid are mentioned, with the inference that they are highly unusual or evidence of polyandry. Pérez (1924), for example, reports three instances of this from his own observations involving *Pleurocrypta porcellanae* Hesse, *P. galatheae* Hesse, and *Athelges lorifera* Hesse. In the light of recent work, these must be interpreted as cases of extra males in process of development before they have realized the full adult state, since all cases, when analyzed, resolve themselves into the stegophryxoid pattern, namely, one adult functional male accompanied by one or more smaller juvenile males or cryptonisci.

It must be concluded, therefore, that it is a general rule in the bopyrids that only one functional male is permitted at a time. Other potential males, temporarily tolerated, are expelled sooner or later. Whether the legitimate male, by virtue of its larger size, actually drives off the others, or whether they are repelled in some more refined manner is a question still to be answered.

Our observations on the question of excess females show that they, like the supernumerary males, are eliminated sooner or later, usually as juveniles. Only one adult female is ever found on an individual host.

One hermit crab, collected August 23, 1946, carried a large ovigerous Stegophryxus and had in addition four juvenile females in various stages of development attached to the abdomen and pleopods. This crab was isolated for daily observations and in less than a week's time the four juvenile females had disappeared. Two other cases of parasitized crabs, each with a juvenile female present in addition to the adult female Stegophryxus, were observed under laboratory conditions. One juvenile persisted from July 24 to August 10 and grew considerably in size before it was eliminated; the other lasted from July 29 to August 11.

It is significant that the lost juvenile females could not be found in the dish in which the crab had been isolated. Perhaps they drop off and are eaten by the crab. It is more probable, however, that they are driven off by the mature male. One finds, on occasion, the mature male wandering about on the outside of the female brood pouch, and it is not unlikely that the male engages in occasional forays over the abdomen of the crab and drives off or destroys the excess females before they reach maturity.

Histological Observations [4]

Four cryptonisci and ten males, the latter selected to form a graded series of sizes ranging from 1.2 mm. to 2.7 mm., were sectioned and studied histologically to determine the sexual nature of the larvae and the organogenesis of the male gonads.

[4] The author gratefully acknowledges the assistance of Cornelius Sharbaugh, T.O.R., who, under our direction, prepared the slides and made the morphological studies on which this portion of the paper is based.

It was found that males of 2 mm.-length and over could be termed "adult"' as judged by the length and development of the testes and the presence of spermatozoa in the vasa deferentia. In such males the reproductive organs are seen as a pair of long tubular masses, beginning in the first thoracic segment, and extending back into the seventh thoracic segment. The testes lie dorso-laterally adjoining the two liver tubes, one on each side of the animal, except for the anterior extremity of each, which occupies a ventro-lateral position in relation to the liver. In the sixth and seventh thoracic segments, the testes become vasa deferentia which open separately to the outside on the ventral surface of the seventh thoracic segment. The beginning of the vas deferens is often dilated to act as a temporary seminal vesicle.

The cells that make up the testis in the anterior-most portion of the organ are all of one type and equally distributed throughout the cross section. Elsewhere, spermatocytes, spermatids, and spermatozoa may be seen arranged in three distinct zones: spermatocytes in the inner zone next to the liver, spermatids in the middle, and spermatozoa in the outer zone (Fig. 4). The cells of the inner and middle zones are grouped into areas or patches, but those of the outer zone extend without interruption the entire length of the testis.

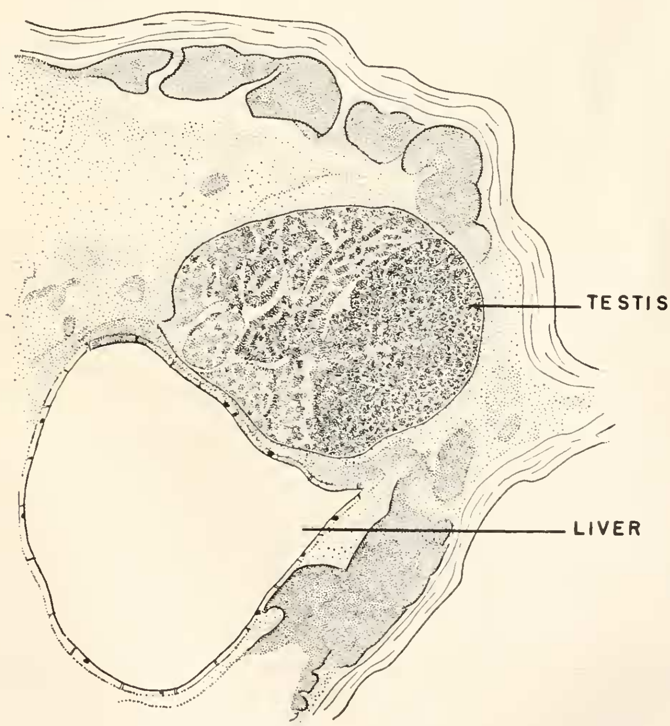

FIGURE 4. Right half of transverse section through the third thoracic segment of a male *Stegophryxus hyptius* measuring 2.7 mm. in length. The testis, dorso-lateral to the liver, shows the characteristic zonal arrangement: spermatocytes in the inner zone closest to the liver, spermatids intermediate in position, and spermatozoa in the outer zone. × 400.

Males of approximately 1.5 mm.-length are essentially similar to the larger males except that the testes are shorter, beginning in the second or third thoracic segment, and the three characteristic zones appear only in the posterior portion. Anteriorly, the testes contain spermatocytes and spermatids, but no spermatozoa.

In the smallest male examined, length 1.2 mm., the testis on the left side was undeveloped; that on the right was short and contained spermatocytes and spermatids only. These occupied a relatively short middle section, with anterior and posterior extremities appearing empty.

None of the four cryptonisci examined, three in cross section and one in longitudinal section, showed either gonads or groups of cells that might be regarded as traces of gonads.

Discussion and Conclusions

The experiments reported on here seem to indicate that the cryptoniscus larvae of Stegophryxus can develop in either of two directions: into males under conditions of parasitism on a female bopyrid, or into females, under conditions of parasitism on a hermit crab. As in the case of Bonellia (Baltzer, 1914), the larvae are apparently indifferent, with both sex potencies. Which potency will be realized depends on the conditions of the environment.

It is evident that the female bopyrid exercises a masculinizing influence on the cryptonisci directly attached to it. This influence does not extend to cryptonisci which are merely in the neighborhood of the female. They receive no male stimulus. To be affected, the cryptoniscus must be in actual contact with the female and perhaps even imbibe her body fluids. Certainly they and the juvenile males receive nourishment from the female, or how else could they grow to increase as much as threefold in size?

Whether nutritive conditions alone provide the masculinizing stimulus, or whether the controlling influence is a specific substance of hormonal nature, is a question requiring further experimental study. Nourishment, as pointed out by Zimmer (1927), is probably the determining factor in the production of females, but for the production of males it seems necessary to assume, as in Bonellia, the transfer of an actual secretion from the body of the female to the larvae that are attached to her, which acts as a specific masculinizing substance.

The sex-determination theory proposed by Giard and Bonnier (1887), namely, that the first larva that invades a particular host transforms into a large female, while the next to come settles on this female and metamorphoses into a dwarf male, is an explanation entirely too simple; but the first assumption, at least, is supported by the results of our experiments. The fate of subsequent comers is less certain. Conceivably, a second cryptoniscus might arrive shortly after the first and also settle down to become an incipient female. There must obviously be a time interval of some days before the first-comer has metamorphosed sufficiently to invite the attention of new arrivals. Let us say, therefore, that the fate of subsequent comers is in no way different from the fate of any cryptoniscus; viz. those that settle directly on a crab become female-determined, while those that settle on a female of their species become male-determined.

One of the objections to the theory of Giard and Bonnier has been the fact that two females are sometimes found on the same host. Thus Hiraiwa (1936) says:

"If the female in the (branchial) cavity makes the later invader into male, why are two females found in one and the same cavity?"

The answer to this objection is now clear. A female does not influence the sex of later invaders unless they settle directly on her body. Should a cryptoniscus settle on the crab, no matter how close in position to a large female, the cryptoniscus will not be affected by this proximity so far as its sex-determination is concerned.

The sex determination theory of Smith (1909) and Goldschmidt (1920) with reference to the Bopyridae requires no discussion. It is eliminated by the facts presented in describing the life cycle of Stegophryxus and has previously been sufficiently criticized by Hiraiwa (1936).

Hiraiwa's own theory, disclaiming as it does differentiation due to environmental factors, is not in harmony with the results of the experiments reported here. His assumption, however, that the free-swimming larvae are not males but are sexually undifferentiated is in agreement with our findings.

Caullery's theory (1941) finds ample confirmation in the results of our experiments. Although the exact experimental verification he hoped for has not yet been realized by us, the converse experiment of transferring cryptonisci from the host to the female bopyrid has yielded satisfactory evidence that the sexes are not fixed from the start.

Coming now to the studies on *Ione thoracica* made by Reverberi and Pitotti (1942) and Reverberi (1947), and their relation to the observations and deductions reported here on *Stegophryxus hyptius,* we find when we tabulate the two for comparison (Table 1) that the same general pattern runs through both. Some, perhaps most, of the differences that do exist are modifications to be expected when comparing species of different genera that differ also in habits and habitat. Thus, since Ione is a branchial parasite, the larvae have the opportunity of settling on the gills of the host (to become female-determined), on the female parasite itself (to become male-determined), or on the skin of the host (likewise to become male-determined). Stegophryxus presents a simpler condition since the female is attached not to the branchiostegite but to the abdomen of the host. Therefore only two substrates are selected for attachment by the larvae: the abdomen of the host or the female herself. If abundant nourishment is the factor that determines females and less abundant nourishment, as Reverberi and Pitotti at first thought, determines males, it is hard to understand why the abdomen of Pagurus should furnish abundant nourishment to the cryptonisci of Stegophryxus while the abdomen of Callianassa should not likewise yield abundant nourishment to cryptonisci of Ione. Later (1947), Reverberi, as a result of further experiments, came to the conclusion that the larvae that attach to the female parasite are masculinized by a sex-determining substance produced by the female rather than by "less abundant nourishment"; but the problem of the so-called "complementary males" on the skin of Callianassa is still very puzzling.

Reverberi's experiments on Ione were made almost exclusively with the older post-cryptoniscid larvae; ours dealt almost entirely with earlier larvae in the cryptoniscus stage. Since the larvae on the body of Callianassa were already presumptive males, his chief experiments were to make females out of them. In Stegophryxus, on the other hand, the cryptonisci on the body of the host are presumptive females, hence our main experiments were to make males out of them. All in all, the two studies complement and illuminate each other. Together they

TABLE 1

A comparison between Ione thoracica and Stegophryxus hyptius with respect to sex-determination. Data for Ione compiled from the papers of Reverberi and Pitotti (1942) and Reverberi (1947)

Ione thoracica	*Stegophryxus hyptius*
1. Adult female lives in the branchial cavity of Callianassa.	1. Adult female lives on the abdomen of Pagurus.
2. Females result from cryptonisci that settle on the gill of the host.	2. Females result from cryptonisci that settle on the abdomen of the host.
3. Females secrete a diffusible substance capable of attracting free cryptoniscus larvae.	3. Same.
4. Cryptonisci that attach to the female become males.	4. Same.
5. Only one adult male is retained.	5. Same.
6. Cryptonisci that attach to the skin of the host became complementary males, capable of replacing the lost male of an adult pair.	6. Cryptonisci that attach to the skin of the host become juvenile females. Replacement of lost males occurs from free cryptonisci only.
7. Juvenile males, removed from adult females, can be transformed into females by placing them on the gills of the host.	7. No evidence that juvenile males can undergo sex reversal, but presumptive male cryptonisci could probably be transformed into females if they left the female and obtained nourishment from the host instead.
8. A widowed male can become female if it succeeds in attaching to the host gill and getting abundant nourishment.	8. It is unlikely that males, once differentiated, could undergo sex reversal.
9. The few comparable experiments reported did not yield results.	9. Presumptive female cryptonisci become males when removed from the host and placed on a female parasite.
10. Evidence supplied in 1947 that differentiated females can undergo sex reversal.	10. No evidence that differentiated females have the capacity for reversal to male.
11. Larvae that engorge host blood directly become females if nourishment is abundant (taken from gills), but become complementary males if nourishment is less abundant (taken from skin of host).	11. All larvae in a position to feed on the host directly become females. Only larvae that attach to the female become males.
12. Reverberi first attributed maleness, when larvae are attached to a female, to "less abundant food," but later (1947) explained it as due to masculinizing substance.	12. Data favors hypothesis of a masculinizing substance produced by the female.
13. Gonad tissue first appears in older post-cryptoniscid forms and the earliest gonad is indifferent.	13. Cryptonisci lack gonads. The earliest gonad in juvenile males is a testis. No data presented on the earliest type of gonad in a juvenile female.
14. Sex-determination is environmental, comparable (with modifications) to that described for Ophryotrocha.	14. Sex-determination is environmental, comparable (with modifications) to that described for Bonellia.

fulfill the expectations of Caullery and reveal a fundamental plan of sex-determination and sex-differentiation, a plan, however, that can be expected to exhibit minor variations when utilized by different genera of Bopyridae.

SUMMARY

Stegophryxus hyptius Thompson, an ectoparasite on the abdomen of the hermit crab *Pagurus longicarpus* Say, seeks the definitive host as a cryptoniscus larva.

The free-swimming cryptonisci are sexually undifferentiated and sexually undetermined. Differentiation follows fixation and is dependent on environmental factors. These conclusions are justified on the basis of the following observations and experiments.

1. Cryptonisci that settle directly on the host develop into females; those that attach to a female bopyrid develop into males.

2. Changes in the color pattern of the cryptonisci following fixation furnish criteria for judging the length of time they have been subjected to a particular environment.

3. Histological examination of the cryptoniscus shows that the gonads are not yet present as recognizable structures.

4. Presumptive female cryptonisci, if removed from the host at an early age and transferred to the brood pouch of a female Stegophryxus, will transform into males.

5. The failure of the converse experiment involving transfer of presumptive male cryptonisci from the female parasite to the host can definitely be laid to experimental difficulties.

6. The factor that determines maleness is a masculinizing substance imbibed with food from the female, but this substance does not act at a distance.

7. Attachment of supernumerary females as well as excess differentiating males is terminated sooner or later so that a particular crab is host to only a single adult female paired with one functional male.

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